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## Abstract

T cell concerned with cell mediated immunity and B cell concerned with humoral antibody are classified by scanning electron microscopy of the surface structure of lymphocytes using E binding lymphocytes and EAC (sensitized sheep erythrocytes treated with complement) binding lymphocytes. For the purpose to elucidate morphological differences between T cell and B cell the scanning electron microscope observations were carried out with the blast forming lymphocytes incubated in the presence of PHA. As a result it has been demonstrated that T cells have short microvilli on the cell surface, but the reason for the difference in the number of the microvilli is unclarified. Even T cells have sometimes long microvilli in the younger stage, they are longer and more slender than those of untreated peripheral B cells.

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## MORPHOLOGIC OBSERVATIONS OF HUMAN T AND B LYMPHOCYTES BY SCANNING ELECTRON MICROSCOPY

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**Abstract:** T cell concerned with cell mediated immunity and B cell concerned with humoral antibody are classified by scanning electron microscopy of the surface structure of lymphocytes using E binding lymphocytes and EAC (sensitized sheep erythrocytes treated with complement) binding lymphocytes. For the purpose to elucidate morphological differences between T cell and B cell the scanning electron microscope observations were carried out with the blast forming lymphocytes incubated in the presence of PHA.

As a result it has been demonstrated that T cells have short microvilli on the cell surface, but the reason for the difference in the number of the microvilli is unclarified. Even T cells have sometimes long microvilli in the younger stage, they are longer and more slender than those of untreated peripheral B cells.

Lymphocytes have many roles and functions in immune responses just as the so-called immune cells and they are classified by their size, maturity, lysosomal enzyme activity, age, specific gravity, etc. (2, 4, 5). Recently, lymphocytes are classified into two subpopulations by their origins; one group is thymus derived cells (T cell), and the other is bone marrow derived cells (B cell). T cells are known to be concerned with cell mediated immunity, and B cells are concerned with humoral immunity. These two subpopulations have been proved in chicken and mouse. Recently, these subpopulations are found to be functionary in man, but there is hardly any morphological report on differences between T and B cells (1, 3, 8, 9, 10, 11, 12, 13, 14, 16, 17).

As markers for T cells of human lymphocytes, human thymus lymphoid tissue antigen and rosette formation to bind sheep erythrocytes are utilized, and as markers for B cells, immunoglobulin determinants and antigen-antibody-complex (1, 3, 10, 11, 12, 13, 14, 16, 17).

In the present observations rosette formation to bind sheep erythrocytes was used as the marker for T cells, and for B cells, sensitized sheep erythrocytes treated with complement (EAC) were used. The observations by the scanning electron microscopy by critical point drying (18) have enabled us to clarify the difference between human T cells and B cells from their morpho-

logical surface structures. In addition, the blast forming cells derived from T cells cultures in the presence of phytohaemagglutinin were observed.

#### MATERIALS AND METHODS

(1) *Purification of lymphocytes in peripheral blood:*

Heparinized peripheral blood (3 ml) was mixed with physiological saline solution (3 ml). This was layered onto the mixture of Ficoll-Conray (3 ml), and the lymphocyte rich layer formed at the interface between plasma and the mixture was removed by pipette after the centrifugation of 2,000 rpm for 30 minutes. The lymphocytes were washed twice in Tris buffer saline solution (tris BSS) by centrifugation at 1,000 rpm for 10 minutes to remove the platelets, and the lymphocytes were suspended in tris BSS to the final concentration of  $1 \times 10^6$  cells/ml.

(2) *Treatment of slide glass with Poly-L-Lysin (PPL):*

PLL (40  $\mu$ g/ml in tris BSS) was dropped onto a slide glass (6×6 mm) which was laid in a small glass vessel (16 mm in diameter), and the slide glass was rinsed twice in tris BSS for 15 minutes or more at room temperature.

(3) *Settlement of lymphocytes to a slide glass:*

One ml of lymphocyte suspension was placed in a small glass vessel immediately, and the lymphocytes were allowed to settle on a slide glass at room temperature for 15 minutes. The slide glass was rinsed in Tris BSS, and one ml of fetal calf serum (FCS) was added into the small glass vessel. Then, lymphocytes were allowed to settle on the slide glass for 15 minutes at room temperature and FCS was decanted.

(4) *Treatment of erythrocyte binding lymphocytes (T cells):*

Sheep erythrocytes (E) were washed twice in Tris BSS and adjusted to  $1 \times 10^6$  cells in FCS. Such an E suspension (2 ml) was added into the small glass vessel containing the lymphocyte-settled slide glass, and the vessel was left standing for one hour at room temperature, followed by storing in refrigerator overnight. Then, the slide glass was taken out from the small glass vessel and washed in phosphate buffer solution (PBS) by pipetting to remove FCS and unreacted erythrocytes from the slide glass (1, 17).

(5) *Treatment of sensitized erythrocytes reacting with complement (B cells):*

According to the method of TACHIBANA (17), one ml of washed erythrocytes (E) ( $1 \times 10^6$  cells/ml in Tris BSS) was sensitized with one ml of 19S rabbit antibody against E stroma at 37°C for 30 minutes. After washing in Tris BSS, one ml of sensitized E ( $1 \times 10^6$  cells/ml) was treated with 1:10 diluted fresh A mouse serum at 37°C for 15 minutes. These were suspended in tris BSS to the final concentration of  $1 \times 10^6$  cells/ml. One ml of EAC suspension was added into the small glass vessel in which lymphocytes-settled slide glass lay after washing the slide glass in tris BSS. The slide glass was incubated in a highly humid incubator at 37°C for 40 minutes and taken out and washed in PBS by pipetting to remove unreacted EAC (1, 17).

(6) *Treatment of blast forming lymphocytes:*

The lymphocytes suspension ( $1 \times 10^6$  cells/ml) was incubated in the mixture of TC-199 solution and inactivated bovine serum (8:2 v/v) added with 1% (v/v) of phytohaemagglutinin (PHA)-M (Difco) at 37°C for 24 hours, 48 hours and 72 hours. The lymphocytes were washed twice in PBS by centrifugation of 1,000 rpm for 10 minutes to remove the tissue culture medium and settled on to the slide glass treated with PLL.

(7) *Treatment of cells for scanning electron microscopy (SEM):*

Each specimen was put into the bath of the fixative fluid (1.2% glutaraldehyde adjusted to pH 7.4 with 0.1 M phosphate buffer) and fixed for one hour. The fixed specimens were immersed in PBS for 10 minutes and dehydrated with a series of graded acetone to be dried by critical point drying method. Carbon and gold-palladium vapors were coated in vacuum on these dry samples.

Observation and photography were done with a scanning electron microscope (JSM-U3 type, Japan Electron Optics Laboratory Co., Ltd., Tokyo), using an accelerating voltage of 25 KV.

## RESULTS

(1) *Untreated lymphocytes:* Untreated lymphocytes of human peripheral blood were classified into 5 types according to the morphology of surface structure by SEM, as follows: lymphocytes which have many short microvilli on the cell surface (Sh) (Fig. 1), lymphocytes which have few short microvilli and whose surfaces are smooth (Sm) (Fig. 2), lymphocytes which have many long microvilli on the surface (L) (Fig. 3), lymphocytes which have few long microvilli and whose surfaces look like wrinkled (W) (Fig. 4) and lymphocytes which have short and comparatively long microvilli on the surface and are considered to be of mixed type of Sh and L (M) (Fig. 5).

Table 1 shows the ratio of these lymphocytes in the peripheral blood. Sh and Sm have the same short microvilli, and there are many types of lymphocytes with varying numbers of microvilli from typical Sh to typical Sm; so, there is no strict distinction between Sh and Sm. The same as Sh and Sm, there are many intermediate types of lymphocytes between L and W, and also there is no strict distinction between the two. The microvilli of type M are shorter than those of L and W but are longer than those of Sh and Sm.

(2) *Rosette forming cells with sheep erythrocytes as the marker of human T cells:* Most of E-rosette forming cells (E-RFC) were Sh and Sm, and negative cells without rosette formation were L and W (Fig. 6). As the ratio of E-RFC is shown in Table 2, 90 per cent of Sh+Sm were positive, and 97 per cent of L+W were negative in rosette formation. Furthermore, M is thought to be T cell because 70 per cent of M were positive in formation (Table 2). From this point of view, it is thought that Sh, Sm and M belong to T cells, and L and W belong to B cells. In untreated lymphocytes, Sh+Sm+M was 76.7

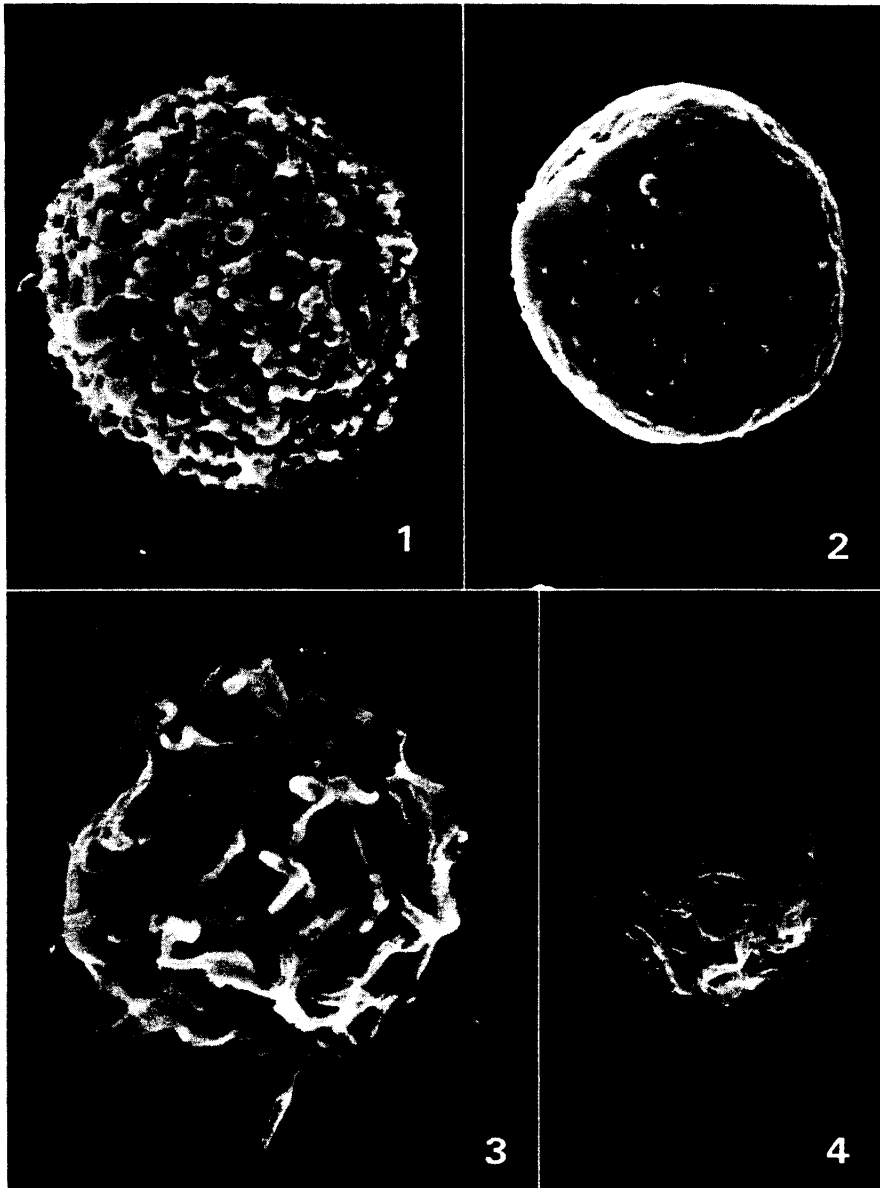


Fig. 1. Lymphocyte having many short microvilli on the cell surface (Sh).  $\times 10,000$

Fig. 2. Lymphocyte having few short microvilli with smooth cell surface (Sm).  $\times 10,000$

Fig. 3. Lymphocyte having many long microvilli on the cell surface (L).  $\times 10,000$

Fig. 4. Lymphocyte having few long microvilli and whose surface appears wrinkled (W).  $\times 6,000$

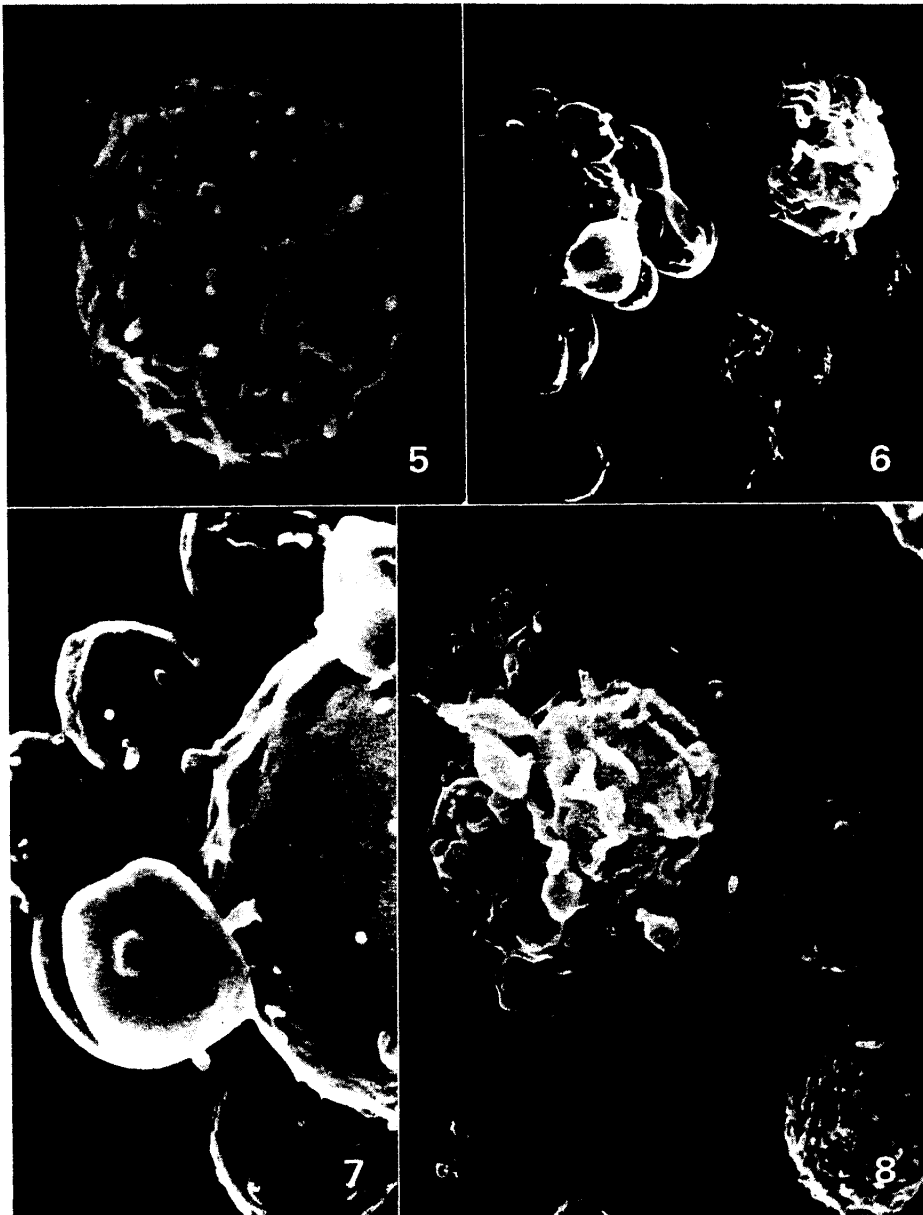


Fig. 5. Lymphocyte having short and comparatively long microvilli on the surface and considered to be of mixed type of Sh and L (M).  $\times 10,000$

Fig. 6. Rosette forming positive and negative cells with sheep erythrocytes.  $\times 5,000$

Fig. 7. Contact phase between lymphocyte and sheep erythrocytes.  $\times 8,000$

Fig. 8. EAC-rosette forming positive and negative cells.  $\times 5,000$

TABLE 1 CLASSIFICATION OF HUMAN PERIPHERAL LYMPHOCYTES

Classification	Number (%)
short microvilli	322 (65.2)
clear surface	38 ( 7.7)
long microvilli	75 (15.2)
wrinkled surface	40 ( 8.1)
mixed type of Sh and L	19 ( 3.8)

TABLE 2 RATIO OF E-ROSETTE FORMING CELLS

Classification	E-RFC (+)	E-RFC (-)
Sh+Sm	300 (90.6%)	31 ( 9.4%)
L+W	3 ( 2.9%)	99 (97.1%)
M	16 (70.0%)	7 (30.0%)

TABLE 3 RATIO OF EAC-ROSETTE FORMING CELLS

Classification	EAC-RFC (+)	EAC-RFC (-)
Sh+Sm	13 (11.9%)	96 (88.1%)
L+W	49 (98.0%)	1 ( 2.0%)
M	0	0

per cent, and L+W was 23.3 per cent; this results coincide with the ratios of T and B cells reported before using several kinds of immunological techniques at the level of light microscope. Fig. 7 shows the contact phase between lymphocytes and sheep erythrocytes but short microvilli heading to the erythrocytes are observed.

(3) *Rosette forming cells with sensitized E reacting with complement as a marker of B cells*: Most of EAC-rosette forming cells (EAC-RFC negative cells were of Sh and Sm (Fig. 8). The ratio of EAC-RFC to be 98% L+W positive and 88% Sh+Sm to be negative means that Sh+Sm belong to T cells, and L+W belong to B cells, the same as in the observation of E-RFC (Table 3). The cause that M could not be found in EAC-RFC may be due to a fewer number of EAC-RFC counted in this instance. Fig. 9 shows the contact phase between lymphocytes and EAC, and it seems that the long microvilli have some role to bind EAC.

(4) *Monocyte*: Monocyte has many long slender microvilli on its surface and is irregular as compared with lymphocytes (Fig. 10).

(5) *Blast forming cells incubated in the presence of PHA*: The lymphocytes incubated for 24 hours have short microvilli and a few long slender microvilli on the cell surface (Fig. 11). The lymphocytes incubated for 48 hours have a few short microvilli and many long microvilli on the surface (Fig. 12). The lymphocytes incubated for 72 hours have long, slender microvilli on the surface (Fig. 13). These long microvilli of incubated lymphocytes differ from those of untreated B lymphocytes in shape. According to the incubation time, the number of long slender microvilli increases.

#### DISCUSSION

Lymphocytes have been classified by the size, maturity, specific gravity, lysosomal enzyme activity, origin, life-span, response against antigen stimu-



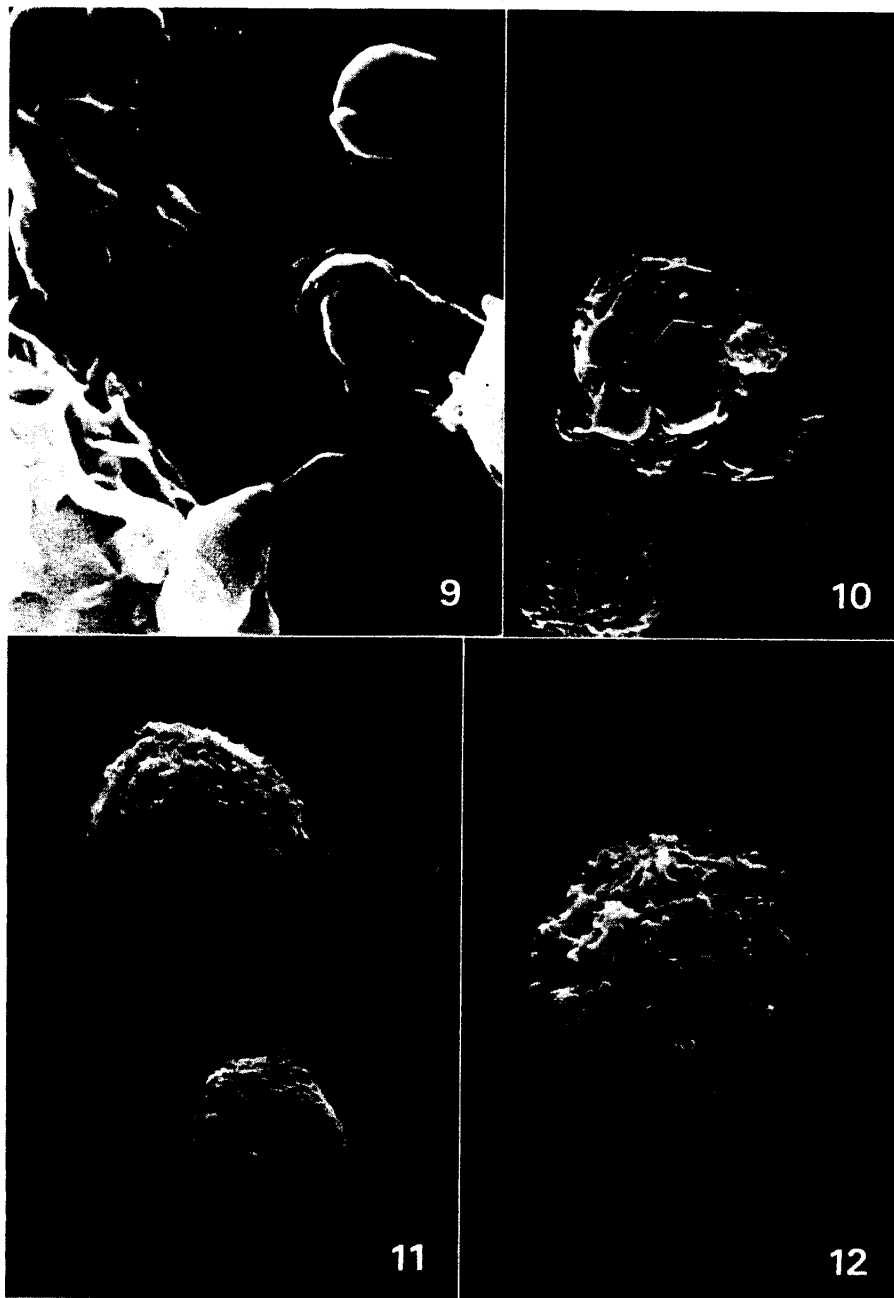


Fig. 9. Contact phase between lymphocyte and EAC.  $\times 8,000$

Fig. 10. Monocyte having many long slender microvilli on its surface.  $\times 5,000$

Fig. 11. Lymphocytes incubated for 24 hours in the presence of PHA.  $\times 5,000$

Fig. 12. Lymphocyte incubated for 48 hours in the presence of PHA.  $\times 5,000$

lation (1, 2, 4, 5). But it has been impossible to classify lymphocytes morphologically into T and B cells using a light microscope or a transmission electron microscope.

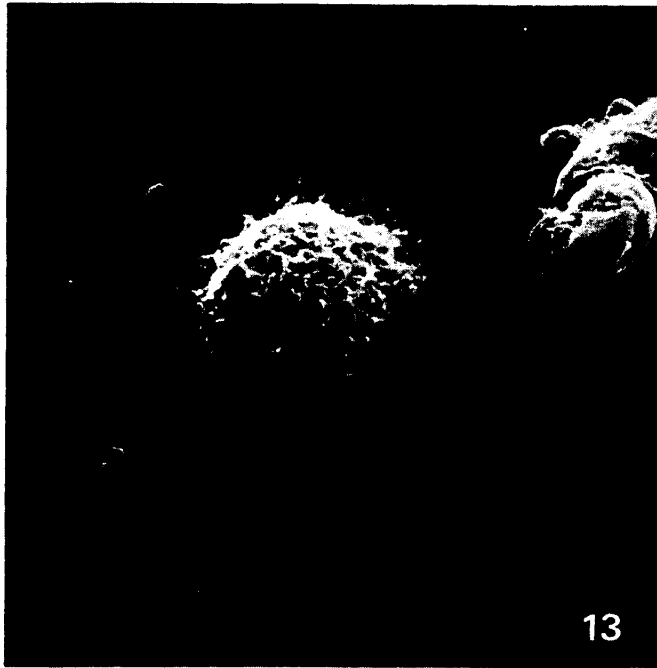


Fig. 13. Lymphocyte incubated for 48 hours in the presence of PHA.  $\times 3,000$

The peripheral lymphocytes are divided into T and B cells according to their origins. Many findings concerning the characters and differences of T, B cells, and immunological markers of human T and B cells are recently reported. As markers of human T cells, human thymus lymphoid tissue antigen and rosette forming with sheep erythrocytes are accepted, and as markers of human B cells, immuno-globulin determinants and complement receptor are known (1, 3, 8, 9, 10, 11, 12, 13, 14, 16, 17).

In this paper, it is clarified by scanning electron microscopy (SEM) that there are differences in the morphological surface structure between T cells which take part in delayed hypersensitivity, transplantation immunity, GVH reaction and cancer immunity, as a host of the so-called cell-mediated immunity, and B cells which are concerned with the humoral antibody secretion (1, 8, 9, 10, 11, 12, 13, 14, 16, 17). Sh and Sm, which are of E-rosette forming type at the same time negative in EAC-rosette forming, belong to T cell. It is difficult to find out strictly the difference between Sh and Sm, and

many lymphocytes which are intermediate type between Sh and Sm can be observed. In spite of the number of the short microvilli on the cell surface, Sh and Sm take up fundamentally an attitude of T cells. It is not clear whether the variety of the number of the microvilli is attributable to the age of lymphocytes, to the difference of the roles concerned with cell-mediated immunity and to the helper of humoral antibody production, or to the other cause.

L and W take up an attitude of B cells in E-RFC and EAC-RFC: so B cells come to have long microvilli on the cell surface. But there is no clear-cut distinction between L and W, and the reason for the difference in these two types of lymphocytes is not clear just as Sh and Sm are in T cells. It is known that B cells adhere readily to glass plate or plastic plate as compared with T cells, but it is thought that it may be attributed to the long microvilli of B cells (1, 10).

M which occupies the intermediate situation of Sh, Sm group and L, W group has both short and long microvilli on the cell surface. The short microvilli are quite identical with those of Sh, Sm group, but the longer ones are shorter than those of L, W group. Furthermore, 70 per cent of M take an attitude of T cell in E-RFC. This seems to imply that M belongs to T cell.

A greater magnification of contact phase of E-RFC and EAC-RFC shows both the short and long microvilli concerned with the binding of E and EAC to lymphocytes. Though LIN *et al.* reported that the primary points of attachment of lymphocytes to red blood cells are microvilli and microvilli serve as the points of attachment of human lymphocytes to antigen, it is thought to be dangerous to assume that only microvilli are concerned with the binding, and the antigen or complement receptor is localized in the microvilli only, because Sm which has a few short microvilli can bind to many sheep erythrocytes (19).

From the difference of the morphological surface structure of the lymphocytes, Sh, Sm, M group is classified to T cell, and L, W group is classified to B cell. The result of the percentage of Sh, Sm, M group coincides with the result of the percentage of T, B cell by light microscopy which has been counted using several kinds of markers up to the present.

LIN *et al.* reported by SEM observation of E-RFC and EAC-RFC using critical point drying method that T cell is smoother with a fewer and shorter microvilli than B cell, but they exclude T cell which has many short microvilli (Fig. 1) and B cell which has few long microvilli (Fig. 4). Furthermore, they state that some T cells have long microvilli and some B cells have short microvilli on the surface, but in our study T cell does not have long microvilli and B cell does not have short microvilli (19).

POLLIACK *et al.* reported surface architecture of human T, B cell ob-

serving human thymocytes, peripheral blood lymphocytes, RFC, cultured cells and chronic lymphocytic leukemia (CLL) by SEM observation using critical point drying method. They use the size of lymphocyte, the number, and the length of microvilli as the identifying points of T and B cells. They report that the microvilli of T cell are found in less than 25 per exposed cell surface and T cell has not so many microvilli; so, it is difficult for them to identify the lymphocytes having many short microvilli (20). By our observations, some T cells have more than 250 short microvilli per exposed cell surface (Fig. 1).

Up to date the distinction of T and B cells is made by using immunophorasis method, rosette forming by sheep erythrocytes, and complement complex, but now it is possible to distinguish the difference of T and B cells by observation of untreated lymphocytes by SEM with critical point drying method.

The blast forming cell by PHA is said to be T cell (15), and these lymphocytes have been shown to have longer and slender microvilli on the cell surface. It means that T cell has short microvilli in the peripheral blood, but in the younger stage of T cell it may have long slender microvilli. The blast forming cell which was incubated for 24 hours has both short and long slender microvilli; the number of the short ones is more than that of the lymphocytes incubated for 48 hours, and the number of long ones is fewer than that of the lymphocytes incubated for 48 hours (Fig. 11, 12). The lymphocytes incubated for 72 hours have long slender microvilli on the cell surface. Almost all of them are long microvilli (Fig. 13).

POLLIACK *et al.* reported that peripheral lymphocytes of 5 cases in 6 patients of CLL were B cell type because 85% of lymphocytes had many long microvilli. As shown in Fig. 13, even T cell has many long microvilli in its younger stage, and the microvilli seem to be longer and more slender than those of normal peripheral B cell. So, it is thought that the lymphocytes of CLL or acute lymphocytic leukemia, which have many long microvilli, may be tumor cells of T cells, and the observation of tumor cells should be done precisely and strictly.

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